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The Behavior of *Staphylococcus Aureus* in Foods Reviewed for the Sanitarian

The behavior of Staphylococcus aureus *has been reviewed with special emphasis for the sanitarian. The topics include sources and characteristics of the organism, food involved in* S. aureus *food-borne outbreaks, characteristics of the enterotoxins, and factors controlling both* wth *and enterotoxin formation in* ds. *The physical and chemical factors which control* S. aureus *growth and enterotoxin formation in foods are: temperature, salt, % brine and water activity, pH and acidity, various chemicals, level of the organism, and competing microflora present in the food. Low temperature (5°C) of storage represents the simplest means for controlling growth of the organism in food. Methods for detection and extraction of enterotoxin from food as well as its quantitation are discussed. In many instances, enterotoxin production is sensitive to extremes of the physical and chemical environment, and growth may occur without concomitant toxin formation.*

Staphylococcus aureus
A coccoid entity
Has plagued the lives of all of us
For an eternity

On skin, in clothes, food, throat, and nose
This minute monster thrives,
What do we need to interpose
To rid it from our lives?

This poem, written by student Michael E. Stiles for the introduction to his Ph.D. thesis 20 years ago (*64*), is still true today. *Staphylococcus aureus* was and is a major cause of food poisoning despite very intensive research during the intervening 20 years. In data compiled by the Centers for Disease Control (CDC) for 1978 and 1979 (the last years for which complete data are available), *S. aureus* was responsible for 26.6% and 32.4%, respectively of the confirmed cases of food poisoning in the United States. For the period 1975 to 1979, *S. aureus* caused 18.8% of the confirmed outbreaks of food-borne illness (*22,23*).

Meats, especially ham, continue to be the major vehicles of transmission. However, foods as diverse as salads, whipped butter, rice balls, Mexican food, and spaghetti have been identified as vehicles (*22,23*). Cream-filled pastries, also a major vehicle, have been the topic of a separate review (*12*). Mayonnaise and mayonnaise-based salads (ham or potato) have acquired a reputation as being the cause of food poisoning (reviewed by Smittle (*63*)). Based on their acid and pH values and salt levels and the "track record" of commercially prepared products, this reputation is scientifically unfounded. Doyle et al. (*27*) in a recent study inoculated chicken and ham salads prepared with and without mayonnaise with *S. aureus* and stored them at 4°, 22°, and 32°C, and found that mayonnaise retarded but did not prevent the growth of *S. aureus* in salads held at 22° or 32°C. They ultimately concluded that mayonnaise contributed to the safety of these foods and along with adequate refrigeration would eliminate any hazard from *S. aureus* in these foods.

The causative agent, *S. aureus*, is part of the normal flora of about 50% of the population, residing on the skin, throat, and nasopharynx (*11*). Any food which comes in contact with humans can become contaminated with the organism, and despite control efforts, adequately processed foods often contain a low number of *S. aureus*. Research from our laboratory (*48*) has indicated that *S. aureus* is killed by the heating step of the frankfurter process. Surkiewicz et al. (*66*) have found that although commercial products immediately upon removal from the smokehouse contain no viable *S. aureus* cells, these same frankfurters peeled, packaged and ready to leave the packing plant contained low numbers of *S. aureus*.

For a food to become an agent o food poisoning, two events mus occur: 1) the food must either be un derprocessed or recontaminated afte processing; and 2) the *S. aureus*-con taining food must be temperatur abused, i.e., held under temperatur conditions favoring growth and toxi production by the organism. Thes CDC reports (*22,23*) indicate that im

[1]*Agricultural Research Service, U.S. Department of Agriculture.*

proper holding temperatures along with inadequate cooking, contaminated equipment, and poor personnel hygiene are the major identifiable contributing factors for foodborne outbreaks. Food processing establishments seldom are implicated as the place where the food was mishandled. In contrast, food service establishments such as restaurants, schools, and camps often are cited.

S. aureus, like *Clostridium botulinum*, produces during its growth in a food a toxic protein that actually causes the food poisoning symptoms. It is possible to have a food in which *S. aureus* cannot be detected by various cultural methods, which yet cause food poisoning. This observation can be duplicated in the laboratory by feeding cell-free culture fluids to human volunteers or animals which subsequently display typical food poisoning symptoms. The time after consumption of the toxin-containing food for first symptoms can range from 30 min to 8 h, with the most occurring in 2-4 h. Symptoms are typical of a gastrointestinal syndrome, with vomiting being the primary response in a majority of cases. Symptoms usually subside within 24 h, and the individual can resume normal activities 1 or 2 days after that. There are seldom any fatalities; however, the disease has been described thusly, "It doesn't kill you, but you wish it would."

The toxin, which is a simple polypeptide protein with a molecular weight of around 30,000, can be differentiated serologically. At present there are six serotypes known, designated SEA to SEE (there are two variations of the C toxin (*10*)). In general, these proteins have no unusual physical-biochemical properties, except for their heat resistance (stability). Tatini (*67*) studied heat treatments commonly used in food processing, including pasteurization (71.7°C for 15 sec) or ultrahigh temperature heating (143.3°C for 9 sec) of fluid whole milk; smoking and heating of cured sausage to 70°-100°C; and heating of Cheddar cheese to 70°-90°C, and found that none were effective for complete destruction of SEA or SED when the toxins were present initially at levels which can be found in foods (0.5-1 μg/100 g). He also found that SEA and SED in whole milk required 15 min heating at 121.1°C for complete inactivation. Thus, toxin production by *S. aureus* cannot be permitted before a food is heat processed since most of the common heat treatments would inactivate the organism, but not any preformed toxin. In addition, the presence of detectable levels of staphylococcal enterotoxin in any food renders the food unfit for human consumption, and the food must not be permitted to enter normal channels. The consumption of as little as 1 μg of enterotoxin has been observed to elicit the food poisoning symptoms in sensitive individuals (*10*). Zehren and Zehren (*75*) analyzed the production of a large cheese processor after certain of its cheeses were implicated in a food poisoning outbreak. Only cheese containing less than 0.3 μg toxin per 100 g cheese (the lower limit of detection) was permitted to enter food channels and no further outbreaks were reported.

Although any of the six serotypes can be produced in foods, strains of *S. aureus* producing SEA are most often isolated from foods incriminated in food-borne illnesses. Interestingly, SEB-producing strains are those studied most often in the laboratory, although SEA-producing strains are those most often seen in cases of food poisoning. The reason for this is that SEB is produced in much higher levels (500-1000 μg/ml of culture fluid) vs. 0.5 to 10 μg/ml for SEA-producing strains. Thus, most data pertaining to conditions in foods such as temperature, pH, salt, and water activity which might control growth and/or toxin production were obtained with SEB-producing strains.

Food poisoning is usually distinguished as either an infection (e.g., *Salmonella, Shigella, Campylobacter*) or an intoxication (*C. botulinum, S. aureus*). With infectious organisms such as *Salmonella*, the presence of any level is considered unacceptable. In contrast, for a toxigenic organism such as *S. aureus*, certain levels are "acceptable" though not considered desirable. This is particularly true for *S. aureus* since any contact the food has with humans 'inoculates' the food with *S. aureus* (see above section on frankfurters). For *S. aureus*, the level (number of cells) is of paramount importance: levels of 10^4/g of food are considered harmless, while levels of 10^7/g are considered unsafe. The region from 10^5 to 10^7/g represent the danger zone and other factors such as the food itself, pH and acid, temperature, time, salt and water activity, and atmosphere become critical in governing toxin production.

The count of *S. aureus* at which detectable toxin is observed varies. Barber and Deibel (*7*) reported an *S. aureus* count of 1×10^7 to 4×10^7/g was the minimal number that produced SEA (lower limit of detection was 0.4 μg/100 g of an inoculated model sausage system), and 8.3×10^8/g for SEB and 2.3×10^8/g for SEE. Gilbert et al. (*32*) reported *S. aureus* colony counts of 7.5×10^5 to 9×10^9/g for food from various food poisoning incidents. Casman and Bennett (*18*) also found *S. aureus* levels of 1×10^6 to 3×10^9/g for food incriminated in food poisonings. An additional factor in food poisoning outbreaks is the observation of variability in that not everyone consuming the food comes down with symptoms. Whether this represents an uneven distribution of *S. aureus* and the toxin or differences in susceptibility of individuals is not known. Further, the amount of food consumed by the different individuals is usually unknown, further complicating the assessment of the risk.

Since *S. aureus* was first found to produce food poisoning, detection and quantitation of the enterotoxins have represented a frustrating problem. Originally, researchers used kittens, monkeys, or human volunteers. Such constitute a cumbersome procedure and preclude analysis of large numbers of samples or accurate quantitation. With the availability of quantities of pure enterotoxins, antisera were prepared and various serological procedures were developed (*5,17,19, 32*). In addition, methods for extraction of the toxins from foods were developed (*5,32*). Extraction of the toxin from the food is a significant feat in itself since the toxin can be present in the food at a level of 0.5 μg/100 g food.

Gilbert et al. (*32*) reported a recovery of enterotoxins A, B, and C from food samples of 20% - 50%. These analytical and serological procedures, although involved and cumbersome, can be scaled up to handle large numbers of samples. A trained 10-person team was able to analyze 4.07 million pounds of cheese from 2,112 vats and clear all but 59 vats for consumption (*75*).

Various modifications of the serological and toxin extraction procedures have been developed. Genigeorgis and Kuo (*29*) developed an affinity chromatographic method using sepharose gel to recover enterotoxin free of interfering food components, followed by microslide gel diffusion plate quantitation. Reiser et al. (*52*) developed an extraction - concentration - digestion procedure for toxin in foods; in conjunction with quantitation by the microslide gel diffusion plate technique, this procedure could complete analyses within 3 days. A recently developed procedure — ELISA (enzyme linked immunosorbent assay) — offers a technique for large scale testing of food extracts containing <1 ng enterotoxins/g food, and can provide this analysis in two working days (*9,28*).

Before discussing how various physical and chemical factors of the food environment influence *S. aureus* growth and toxin production, a brief discussion of metabolism is presented. Metabolism is considered to be either primary or secondary. Primary metabolism is any of the reactions or products which are necessary for the primary growth of the microorganism and generally occurs during the log phase of growth. Examples of primary metabolism and metabolites are lactic acid from lactic acid bacteria and ethanol and carbon dioxide from yeast. Secondary metabolism is any of a series of reactions and products which are formed after primary (log) growth of the microorganisms and whose exact metabolic function is not known with certainty. Examples include antibiotic and aflatoxin formation by molds. At present, SEB appears to be a secondary metabolite (formed after primary growth of the culture), while SEA seems to be a primary metabolite (formed as the culture is growing logarithmically) (*15,16*). There are some problems with these distinctions since SEA is formed in much lower quantities, making quantitation at the lower limit of detection difficult. What is important in this discussion is that many of the factors to be mentioned below will affect the primary rate of growth of *S. aureus* and these will affect the amount of SEA formed. Since SEB is considered to be a secondary metabolite, there can and should be conditions in a food which will permit growth of the organism without concomitant toxin production.

As mentioned above, the influence of various physical and chemical factors of the food environment on growth and toxin production by *S. aureus* have been studied quite extensively. Low temperature is one of the most important and represents, in a sense, an additive or process which requires no approval and is not regulated. Low temperature (below 5° C) holding of a food is one of the easiest ways to limit the proliferation of most pathogens. (Exceptions are the two relatively rare food-borne pathogens: *Clostridium botulinum* type E and *Yersinia enterocolitica*.)

Angelotti et al. (*1*) inoculated *S. aureus* into sterile custard, chicken a la king, and ham salad and observed small increases in *S. aureus* counts for custard and chicken a la king at 44°F and above, while there was no change in the number of *S. aureus* in the ham salad held over a 5-day period at temperatures of 44°-10°C. Goepfert and Kim (*33*) inoculated ground beef with *S. aureus* and observed no increase in numbers during a 5-day storage at 12.5°C. Doyle et al. (*27*) found no growth of *S. aureus* in meat salads prepared with and without mayonnaise when held at 4°C. There is some variation observed in different foods, but *S. aureus* does not appear to grow below 41°F.

The temperature minimum for inhibiting toxin production by the organism is not as restrictive as for growth inhibition. Donnelly et al. (*26*) inoculated pasteurized milk with an SEA-producing strain of *S. aureus* (either 10^4 or 10^6/ml) and held the milk at various temperatures. Neither growth nor toxin production was observed after 168 h at 10°C. However, Scheusuer and Harmon (*54*) inoculated vanilla pudding with 10^5 *S. aureus* per g and observed formation of SEA, SEB, SEC, and SED at 10°C. Genigeorgis et al. (*30*) observed SEB production in ham stored anaerobically at 10°C, though not all samples supported toxin production. Any extended exposure of a food to temperature >10°C creates the potential for a food poisoning outbreak. Even though the amount of toxin formed decreases dramatically as the holding temperature decreases to the 10°C minimum, the production of even small quantities of toxin (1-5 μg) is to be avoided since it is known that less than 1 μg of enterotoxin per 100 g of food is sufficient to produce food poisoning symptoms in individuals (*10*). The emitic dose of the various enterotoxins for monkeys does vary, ranging from 5 μg/monkey for SEA and SEB to 20 μg for SED. Corresponding data for humans are not available, though as indicated, 1 μg has been observed to elicit symptoms in humans (*10*).

With respect to refrigeration and low temperature holding of heated foods, particular attention must be devoted to the size of the containers and the rate of cooling. Shallow pans and trays serve as better containers compared to a single large pot. As an example, in a food service establishment, chicken cooked for pot pies is removed from the bone by hand; if placed back in the same large cooking container and refrigerated this chicken can provide an excellent opportunity for an *S. aureus* food poisoning outbreak. In contrast, use of a number of shallow containers will minimize the time the boned chicken is in the temperature range of 45°-10°C, the temperature range for toxin production by *S. aureus*.

Shifting from the minimum to the maximum, *S. aureus* growth and toxin production can be prevented at high holding temperatures. Angelotti et al. (*2*) observed the growth of *S. aureus* in sterile custard held at 45.5°C, but not at 46.7°C, and in sterile chicken a la king at 44.4°C, but not 45.5°C; there was no growth in sterile ham salad held at 44.4°C and above. Holding at temperatures above the maximum was lethal to the organism.

A particular interesting temperature

phenomenom was studied by Hughes and Hurst (*34*) and Hurst et al. (*35*), and while at present it has not found application in a food system, the potential is there. These investigators found that salts such as NaCl, $MgCl_2$, and KCl and sugars such as sucrose and glucose raised the maximum temperature of growth and toxin production of *S. aureus* by 2°C. Though only a relatively small increase in maximum temperature was observed, any food containing these solutes which is held close to the maximum growth temperature of *S. aureus* could become a cause of food poisoning. Since the solutes exhibiting this protection effect are often found in foods, any food which could contain *S. aureus* should be held substantially above the 45°C observed maximum temperature for *S. aureus*.

As mentioned previously, any temperature above the maximum temperature for growth (116°F or 47°C) is lethal for the organism. The greater the temperature above the maximum, the faster the killing. Angelotti et al. (*3*) had reported D values (time to bring about a one log kill (90% destruction)) ranging from 61 min at 54.4°C to 0.64 min at 65.5°C for *S. aureus* 196E in chicken a la king. Similar values were obtained in custard and with a second strain. Stiles and Witter (*65*) studied the effect of pH of a phosphate buffer heating medium on heat resistance and observed lower D values (faster killing) as pH decreased from 7.5 to 4.5. Walker and Harmon (*73*) reported greater resistance (higher D values) of *S. aureus* heated in skim milk and Cheddar cheese whey compared to heating in phosphate buffer or whole milk. Work in our own laboratory had indicated that salt (5% NaCl in distilled water) can protect *S. aureus* from both injury and death, and that the temperature of heating must be raised more than 6°C to bring about killing of the organism (*57*). Though various food components can protect from thermal injury (*58*) and increase the amount of heat needed (raise the temperature) to kill *S. aureus*, the thermal processes used for most food appear adequate to destroy any *S. aureus* present. *S. aureus* is killed by the milk pasteurization treatments (62.8°C for 30 min or 71.7°C for 15 s) (*45*); by the 'cook' given semi-preserved canned hams (heated to an internal temperature of 71.1°C) (*45*); and by the smokehouse heating schedule used in the processing of frankfurters (heated to an internal temperature of 71.1°C) (*48*). Castellani et al. (*21*) reported that a temperature of 73.9°C in the center of stuffing was sufficient to kill *S. aureus* during turkey roasting. While these data are mostly for meat products, these temperatures should be applied to all foods. Food preparers should be especially careful and measure the temperature at the coldest part of the food, usually at or near the geometric center.

As mentioned above and will be often repeated throughout this review, *S. aureus* is an extremely salt-tolerant bacterium. This characteristic is the basis for the media originally developed to isolate *S. aureus* from foods: 7½% NaCl. This salt tolerance is also why *S. aureus* grows so well in many foods, especially ham and other cured meats. In fact, addition of salt to many foods selects against other bacteria while selecting for *S. aureus*. An item of particular interest can be mentioned here. There was and is considerable concern over the addition of nitrite to cured meats. One of nitrite's maior functions in cured meats is to prevent the growth of *C. botulinum*. In order to eliminate the need for nitrite to control *C. botulinum* in cured meats, it was proposed to add enough salt to give meat products with 10% brine (water activity of 0.92) (*46*). Although *C. botulinum* would be controlled, the conditions established would be just at the limit for toxin production by *S. aureus*. One problem would be substituted for another!

Before reviewing the effect of salt on *S. aureus*, a new concept regarding salt levels in foods must be introduced. This is brine concentration (% brine), a measure of salt concentration in the aqueous phase of foods which equals

$$\frac{\text{grams salt}}{\text{grams salt} + \text{grams water}} \times 100$$

While water activity (to be mentioned below) is a better absolute indicator of whether or not a given microorganism will or will not grow in a particular food, brine concentration is more readily obtainable and applicable to foods. Riemann et al. (*53*) in their study on factors influencing growth and toxin production by *S. aureus* in semi-preserved meats, determined that very few semi-preserved meat products available to American consumers

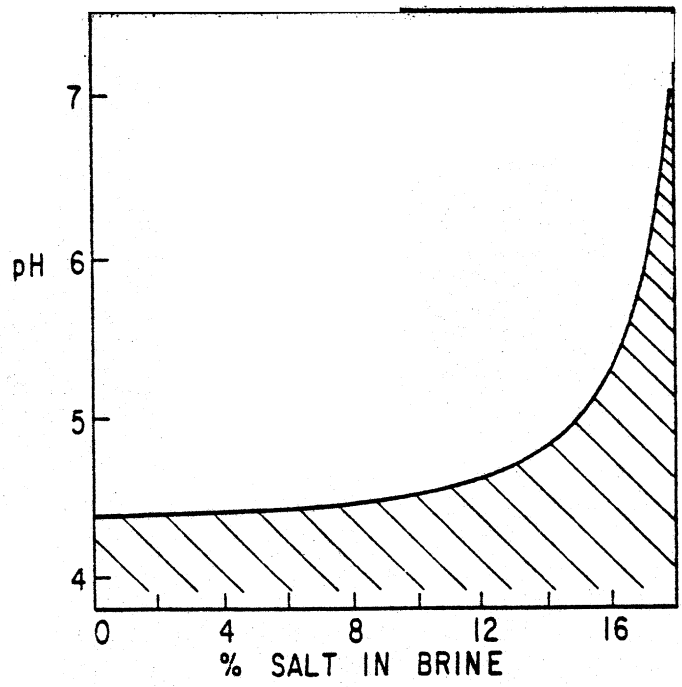

Figure 1. *Effect of pH and % brine on growth of* Staphylococcus aureus *in foods and culture media. Cross-hatching indicates food or medium in which combined pH and % brine prevented growth of the organism. Redrawn from Riemann et al.* (53).

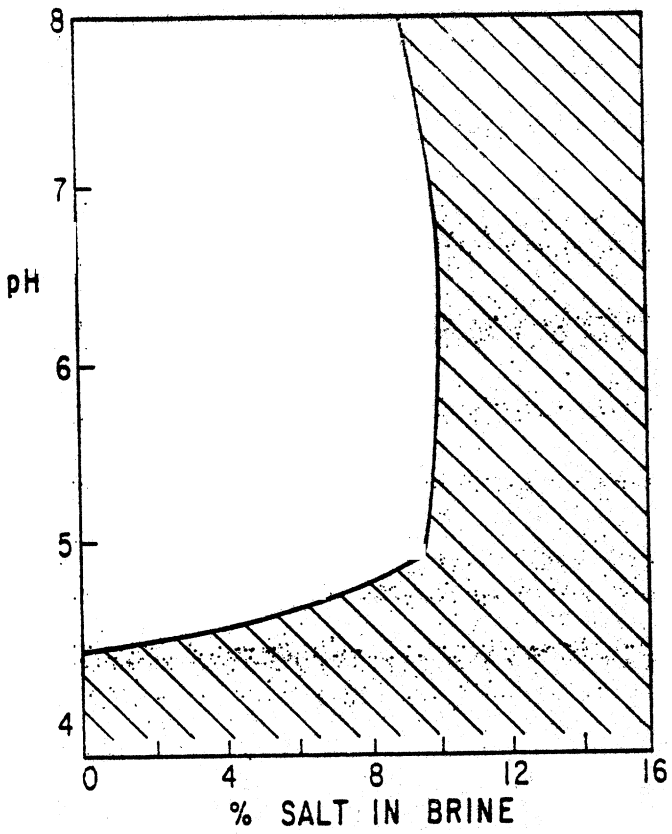

Figure 2. *Effect of pH and % brine on enterotoxin production by* Staphylococcus aureus *in food and culture media. Cross-hatching indicates food or medium in which combined pH and % brine prevented enterotoxin production by the organism. Redrawn from Riemann et al. (53).*

have sufficiently high brine concentrations to inhibit *S. aureus* completely. They also considered pH as a factor that had some influence on growth and toxin production and these data (taken from Riemann et al., (*53*)) are presented in Figures 1 and 2. These figures are composites from both food and culture media. Any product or culture medium whose pH and brine concentration fall in the upper left portion of the graph would support either growth (Figure 1) or toxin production (Figure 2). Any product whose pH and brine level fell outside the upper left quadrant (cross-hatched area) generally would not support growth or toxin production.

The minimum pH for either growth or toxin production is around 4.5. Toxin formation is more sensitive to brine level than is growth, with 10% brine being the maximum at which toxin can be formed. A last point should be made - these data generally were obtained under conditions under which other parameters (temperature, atmosphere, etc.) were ideal and with a starting inoculum of 1×10^7/g or ml. If any of these conditions becomes nonideal, the limiting pH and brine concentrations combination is changed, with the pH value increased and brine level decreased.

The specific acid used in the preparation of various foods can influence their ability to restrict the growth of *S. aureus*. Using pasteurized milk, Minor and Marth (*39*) found a gradation in pH values with different organic and inorganic acids. To achieve a 99% or 2 log value reduction in growth over a 12-h period, a final pH value of 5.2 was required for acetic, 4.9 for lactic, 4.7 for phosphoric and citric, and 4.6 for hydrochloric acid.

Extremes of many growth parameters often are not conducive to either growth or survival. Temperatures above the maximum for growth are lethal; pH values below the minimum for growth also are lethal. Minor and Marth (*40*) observed decreased viability for *S. aureus* inoculated into various fermented dairy products: cultured buttermilk, sour cream, and yogurt, with corresponding pH value ranges of 4.1-4.4, 4.3-4.4, and 3.7-4.1, respectively.

Metabolically, *S. aureus* is classified as a facultative anaerobe which will grow more rapidly and abundantly under aerobic conditions (*13*). Most of the studies on the effect of atmosphere have been performed on culture systems. Woodburn et al. (*74*) observed that shaking during incubation increased greatly the production of SEA, SEB, and SEC as compared to static incubation.

Some investigators have suggested dissolved oxygen (DO) as a better parameter to describe the influence of oxygen on growth and enterotoxin formation. Carpenter and Silverman (*15*), in their study of SEB synthesis, found growth best at 100% DO, but no SEB was synthesized. When DO decreased to 50%, growth decreased and there was a marked increase in SEB formed. Maximum SEB was formed at 10% DO. In contrast, formation of SEA appears more directly related to the amount of growth by the producing strain. In a further study, these investigators (*16*) did not find an optimum DO for SEA and concluded that SEA formation is independent of DO.

As found in culture media, enterotoxin formation in foods is more abundant under aerobic than anaerobic conditions. Genigeorgis et al. (*31*) studied enterotoxin B production in hams held aerobically at 10°, 22°, and 30°C. They found better toxin production at 30°C than at 22° or 10°C, but toxin was detected after 2 weeks in samples held at 10°C. In addition, toxic hams appeared normal even after 2 months storage at 10°C. In their study of *S. aureus* in Canadian bacon, Thatcher et al. (*70*), found that in bacon samples held at 37°C, enterotoxin was found in samples packed under atmospheres of air, 5% CO_2+95% O_2, and nitrogen. Only a small amount of enterotoxin was formed under vacuum. It should be noted in this study that the bacon incubated in air or CO_2+O_2 mixture was obviously spoiled. Cooked peeled prawns, often involved in food poisoning outbreaks in England, were inoculated with SEB-producing strains

of *S. aureus* and held at temperatures between 22° and 36°C in either air or 95% N_2 + 5% O_2. Depending upon inoculum level, aerobically incubated prawns contained SEB after 7 days at 26°C and higher. The amount of SEB and rate of its production were best at >30°C. There was no SEB produced under the N_2+CO_2 atmosphere. All SEB-containing prawns were organoleptically spoiled at the time SEB was detected. Using a model sausage system, Barber and Deibel (*7*) observed detectable SEA after 24 h in 10%, 15%, and 20% oxygen atmospheres, and after 48 h in 5% oxygen. No SEA was produced under anaerobic conditions. Bennett and Amos (*8*) inoculated sausage, hamburger, and turkey sandwiches with enterotoxigenic *S. aureus* and stored them under nitrogen at 8°, 12°, and 26°C. At 8° and 12°C, none of the sandwiches became toxic after 31 days storage. At 26°C, sausage and hamburger sandwiches were toxic at 2 and 4 days, respectively, while remaining organoleptically acceptable. Turkey sandwiches did not support sufficient *S. aureus* growth to yield detectable amounts of toxin at any temperature. Growth and toxin formation can occur in a wide variety of foods and under the different atmospheres food might be held. In addition, toxin-containing foods often retain their organoleptic properties and probably would be eaten and cause food poisoning.

Implicit in the above discussions of how the various factors affect growth and toxin production is the interrelationship between all these factors, the starting number of *S. aureus* and the presence or absence of competing bacteria. Pathogens are generally considered to be poor competitors. Ham, often a vehicle for *S. aureus* food poisoning, provides a seemingly excellent substrate because the heat processing kills virtually all of the normal flora of the meat and the brine content limits the recontaminating organisms to those which can grow to the limited number of salt tolerant organisms such as *S. aureus*. Thus, recontamination by even small numbers of *S. aureus* followed by temperature abuse would have a strong potential for a food poisoning episode.

Low (ca 100/g) number of *S. aureus* have been shown to grow to sufficient numbers to yield detectable enterotoxin. Lee et al. (*38*) observed SEA, SEB, and SEC in macaroni dough inoculated to give a starting count of ca 50 *S. aureus*/g. Toxin was formed at both 25° and 35°C. Casman et al. (*20*) inoculated the surface of cooked and raw (low bacterial count) pork and bacon with ca 250 *S. aureus*/g, and observed both growth and SEA formation at 30°C. Depending upon salt, nitrite, and holding temperature, Genigeorgis et al. (*31*) observed both growth and SEB and SEC production in cooked pork, beef, and ham in conjunction with a starting count of 10^3/g.

Similar effects have been noted in dairy products. Ikram and Luedecke (*37*) inoculated whole milk, skim milk, whipping cream, and half and half with 10^3 *S. aureus*/g and held them at either 37° or 22°C. Growth and SEA formation occurred at 37°C, with little growth and no SEA formation occurring at 22°C. When pasteurized milk used to manufacture Cheddar cheese was inoculated with 5-80 *S. aureus*/ml and an incative starter culture, SEA was detected in cheeses ripened at 11°C (*36*). Inactivated starter cultures is one of the key factors in permitting toxin formation in these products. As previously mentioned, Zehren and Zehren determined that an active starter culture is a simple and extremely effective means of controlling *S. aureus* during Cheddar cheese manufacture (*76*).

These few examples illustrate that *S. aureus* is capable of growing and producing toxin in foods when initially present at levels of 10^2-10^3/g or ml. The normal microflora of foods usually inhibit *S. aureus* growth and subsequent enterotoxin formation. This is particularly true of various lactic acid bacteria and is seen in the above example of Cheddar cheese production. Bacteria other than lactics also can influence *S. aureus* in foods. For example, Peterson et al. (*49*) observed that the naturally occurring mixed bacterial flora of mesophiles and psychrophiles in frozen chicken pot pies and macaroni and cheese dinners prevented the growth of added *S. aureus* during the defrosting of these foods. However, while *S. aureus* growth can be restricted in competitive situations in foods, with the exception of lactic acid bacteria fermented foods, this method cannot be relied on as an adequate procedure for protecting foods from *S. aureus* hazards.

In addition to salt and pH/acids, many other compounds (chemical food ingredients or additives) can restrict *S. aureus* growth and toxin formation in foods. The list of these compounds includes sodium nitrite (cured meats), potassium sorbate (bacon, (*50*)), glucono-delta lactone + citric acid (sausage, (*24*)), phenolic-type antioxidants (dry sausage, (*51*)), and glycerol monolaurate (model sausage product (*61*)). Work from our own laboratory (*61*) and others have indicated that these compounds are more effective under anaerobic conditions, i.e., less compounds is needed to achieve the same level of inhibition under anaerobic conditions. Smoke, applied as part of the normal processing given a product such as pepperoni, can also restrict *S. aureus* (*69*).

Recently, studies have shown that many of the unit operations of food processing such as heating, acidification (fermentation or pickling), and freezing can stress or injure rather than kill microorganisms (*14,25,56, 60*). This topic has previously been reviewed for the sanitarian (*62*). Of interest and concern to the sanitarian is the observation that injured (stressed) cells are incapable of forming colonies on the selective media often used to isolate various microbial groups from foods. These media contains dyes, various chemicals, and sodium chloride (for *S. aureus*) as selective agents.

Restricting the rest of the discussion to *S. aureus*, use of a salt-containing (7½% NaCl) medium to quantitate *S. aureus* in a heat processed food could lead to an inaccurate estimation of the bacteriological quality of the food and/or false evaluation that the food was adequately heated. The inability of heated or other stressed cells to grow on the salt-containing media typically used to isolate *S. aureus* from

foods has led to the development and use of NaCl containing media, e.g., Baird-Parker agar (*4*), both for direct plating and for most probable number determination of *S. aureus* from processed foods.

In addition to the concern of not detecting injured *S. aureus* in a processed food, and thus not know the quality of the food, there is the potential that the injured cells can recover normal cellular functions and then produce enterotoxin in the food. At this point, we are not aware of any documented food poisoning outbreak due to injured *S. aureus* which have recovered in a food and then produced toxin. However, work from our laboratory (*47*) has demonstrated that injured *S. aureus* can repair (regain salt tolerance) on a model food system agar (toxin production by these cells was not determined). Heat injured *S. aureus* repaired on a ground beef agar (GBA), GBA containing 2½% NaCl, 5% KCl, 15% glycerol, 30% sucrose, 400 ppm nitrite, 500 ppm ascorbate, and lactic acid down to pH 5.5. Repair also occurred at temperatures of 20° to 45°C, also on frankfurters and chili beef soup agars, but not on pepperoni or Lebanon bologna agars. Since many food processing operations can injure *S. aureus*, and since *S. aureus* can repair under food conditions, precautions should be exercised to detect injured *S. aureus* if they are present. The recommended Bacteriological Analytical Manual procedure, Baird-Parker agar, will detect heat injured cells (*33a*). In addition, food preparations should insure the complete destruction of *S. aureus* and avoid conditions which would just injure the organism.

As indicated above, salt, in high enough concentrations, can restrict the growth of *S. aureus*. Salt as well as other solutes limits or completely inhibits the growth of various microorganisms by restricting the amount of water available to the cells. The water available to the cells is generally expressed as water activity (A_w) which is defined by the equation:

$$A_w = \frac{P}{P_o}$$

where P is the vapor pressure of the test (unknown) solution or food and P_o is the vapor pressure of water at the same temperature. Further, $A_w \times 100$ is the equilibrium relative humidity of the solution or food. Increasing the amount of salt or other solutes present decreases the A_w of the growth medium or food. For a more detailed discussion of A_w, consult the classic paper by Scott (*55*), and also Troller (*71*) and Troller and Stinson (*72*) for reviews of the effect of water relations among various food-borne pathogens, expecially *S. aureus*. For Tables of A_w or various foods and A_w limits of various microorganisms and groups of microorganisms (food spoilage organisms as well as pathogens), Banwart (*6*), Tables 4.6 and 4.8, respectively should be consulted.

This review was not intended to be a comprehensive one dealing with *S. aureus* in foods, but rather, was restricted to those areas of interest to sanitarians. In this last section, the reader is referred to the literature for additional information. Here again, the following is not comprehensive. Minor and Marth, in addition to their book, "Staphylococci and Their Significance in Foods" (*45*), wrote a very readable series of papers entitled "*Staphylococcus aureus* and staphylococcal food intoxications. A review," in which they discussed: A) the nature of the organism, its characteristics, physiology, and isolation (*41*), B) enterotoxins and epidemiology (*42*), and staphylococci in C) dairy foods (*43*), and D) meat, bakery products, and other foods (*44*).

Riemann et al. (*53*) have reviewed the various mechanisms for controlling *S. aureus* in semi-preserved (non-shelf stable or keep-refrigerated) meat products and concluded that low temperature storage rather than low pH or high brine (salt) levels is an extremely functional way to preserve these products. The pH and brine levels needed to inhibit *S. aureus* growth and toxin production would yield meat products with limited appeal to the current American consumer. Further, since American consumers are interested in reducing their salt intakes, salt is not a viable means of restricting *S. aureus* in meats.

Bryan (*11*) provided an earlier review of many of the same topics considered in this review, though from a different point of view. Tatini (*68*) has summarized the effects of various food environments on *S. aureus* growth and enterotoxin formation. Bergdoll (*10*) has reviewed the area of *S. aureus* intoxication in depth, drawing upon much of his own research and experience at the Food Research Institute (University of Wisconsin, Madison). His work provides an up-to-date and readable discussion of the area. The recent review by Smith et al. (*59*) provides an in depth discussion of environmental factors controlling enterotoxin synthesis, especially in foods.

REFERENCES

1. Angelotti, R., M. J. Foster, and K. H. Lewis. 1961. Time-temperature effects on salmonellae and staphylococci in foods. I. Behavior in refrigerated foods. Amer. J. Pub. Health 51:76-83.
2. Angelotti, R., M. J. Foster, and K. H. Lewis. 1961. Time-temperature effects on salmonellae and staphylococci in foods. II. Behavior at warm holding temperatures. Amer. J. Pub. Health 51:83-88.
3. Angelotti, R., M. J. Foster, and K. H. Lewis. 1961. Time-temperature effects on salmonellae and staphylococci in foods. III. Thermal death time studies. Appl. Microbiol. 9:308-315.
4. Bacteriological Analytical Manual. 1978. Food & Drug Administration, Bureau of Foods, Division of Microbiology. Published by AOAC, Washington, DC 20044.
5. Baird-Parker, A. C. 1970. Detection of *Staphylococcus aureus* and its food poisoning toxins. *In* Proceedings SOS 70-Third International Congress of Food Science and Technology, Washington, DC, August 9-14, 1970, pp. 672-675. Published by IFT, 1971.
6. Banwart, G. J. 1979. Basic Food Microbiology, AVI Publishing Co., Inc., Westport, CT.
7. Barber, L. E., and R. H. Deibel. 1972. Effect of pH and oxygen tension on staphylococcal growth and enterotoxin formation in fermented sausages. Appl. Microbiol. 24:891-898.
8. Bennett, R. W., and W. T. Amos. 1982. *Staphylococcus aureus* and toxin production in nitrogen-packed sandwiches. J. Food Prot. 45:157-161.
9. Berdal, B. P., O. Olsvik, and T. O. Omland. 1981. A sandwich ELSIA method for detection of *Staphylococcus aureus* enterotoxins. Acta Path. Microbiol. Scand. Sect. B 89:411-415.

10. Bergdoll, M. S. 1979. Staphylococcal intoxications. *In* Food-borne Infections and Intoxications. H. Riemann and F. L. Bryan, eds. Academic Press, New York, NY.
11. Bryan, F. L. 1968. What the sanitarian should know about staphylococci and salmonellae in nondairy products. J. Milk Technol. 31:110-116.
12. Bryan, F. L. 1976. Public health aspects of cream-filled pastries. A review. J. Milk Food Technol. 39:289-296.
13. Buchanan, R. E., and N. E. Gibbons. 1974. Bergey's Manual of Determinative Bacteriology. 8th Ed. The Williams and Wilkins Co., Baltimore, MD.
14. Busta, F. F. 1976. Practical implications of injured microorganisms in foods. J. Food Technol. 39:138-145.
15. Carpenter, D. F., and G. J. Silverman. 1974. Staphylococcal enterotoxin B and nuclease production under controlled dissolved oxygen conditions. Appl. Microbiol. 28:628-637.
16. Carpenter, D. F., and G. J. Silverman. 1976. Synthesis of staphylococcal enterotoxin A and nuclease under controlled fermentor conditions. Appl. Environ. Microbiol. 31:243-248.
17. Casman, E. P. 1958. Serologic studies of staphylococcal enterotoxins. Public Health Reports 73:599-609.
18. Casman, E. P., and R. W. Bennett. 1965. Detection of staphylococcal enterotoxin in foods. Appl. Microbiol. 13:181-189.
19 Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. E. Stone. 1969. The microslide gel double diffusion test for the detection and assay of staphylococcal enterotoxins. Health Lab. Sci. 6:185-198.
20 Casman, E. P., S. W. McCoy, and P. J Brandly. 1963. Staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 11:495-500.
21. Castellani, A. G., R. R. Clarke, M. I. Gibson, and D. F. Meisner. 1953. Roasting time and temperature required to kill food poisoning microorganisms introduced experimentally into stuffing in turkey. Food Res. 18:131-138.
22. Centers for Disease Control. 1981. Foodborne Disease Outbreaks. Annual Summary 1979. Issued April, 1979.
23. Centers for Disease Control. 1981. Foodborne Disease Outbreaks. Annual Summary 1978. Revised, Reissued February, 1981.
24. Daly, C., M. LaChanc, W. E. Sandine, and P. R. Elliker. 1973. Control of *Staphylococcus aureus* in sausage by starter cultures and chemical acidulation. J. Food Sci. 38:426-430.
25. Demchick, P. F., S. A. Palumbo, and J. L. Smith. 1982. Influence of pH on freeze-thaw lethality in *Staphylococcus aureus*. J. Food Safety 4:185-189.
26. Donnelly, C. B., J. E. Leslie, and L. A. Black. 1968. Production of enterotoxin A in milk. Appl. Microbiol. 16:917-924.
27. Doyle, M. P., N. J. Bains, J. L. Schoeni, and E. M. Foster. 1982. Fate of *Salmonella typhimurium* and *Staphylococcus aureus* in meat salads prepared with mayonnaise. J. Food Protect. 45:152-156.
28. Freed, R. C., M. L. Evenson, R. F. Reinser, and M. S. Bergdoll. 1982. Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. Appl. Environ. Microbiol. 44:1349-1355.
29. Genigeorgis, C., and J. K. Kuo. 1976. Recovery of staphylococcal enterotoxin from foods by affinity chromatography. Appl. Environ. Microbiol. 31:274-279.
30. Genigeorgis, C., H. Riemann, and W. W. Sadler. 1969. Production of enterotoxin B in cured meats. J. Food Sci. 34:62-68.
31. Genigeorgis, C., M. Savonkidis, and S. Martin. 1971. Initiation of staphylococcal growth in processed meat environments. Appl. Microbiol. 21:940-942.
32. Gilbert, R. J., A. A. Wieneke, J. Lanser, and M. Simkovicova. 1972. Serological detection of enterotoxin in foods implicated in staphylococcal food poisoning. J. Hyg. Camb. 70:755-762.
33. Goepfert, J. M., and H. U. Kim. 1975. Behavior of selected food-borne pathogens in raw ground beef. J. Milk Food Technol. 38:449-452.
33a. Gray, R. J. H., M. A. Gaske, and Z. J. Ordal. 1974. Enumeration of thermally stressed *Staphylococcus aureus* MF31. J. Food Sci. 39:844-846.
34. Hughes, A., and A. Hurst. 1980. The effect of NaCl on the upper temperature limit for growth of and enterotoxin by *Staphylococcus aureus*. Can. J. Microbiol. 26:507-510.
35. Hurst, A., A. Hughes, and R. Pontefract. 1980. Mechanism of the temperature protective effect of salts on *Staphylococcus aureus*. Can. J. Microbiol. 26:511-517.
36. Ikrahem, G. F., D. R. Radford, A. K. Badlock, and L. B. Ireland. 1981. Inhibition of growth of *Staphylococcus aureus* and enterotoxin production in Cheddar cheese produced with induced starter culture failure. J. Food Protect. 44:189-193.
37. Ikram, M., and L. O. Luedecke. 1977. Growth and enterotoxin A production by *Staphylococcus aureus* in fluid dairy products. J. Food Protect. 40:769-771.
38. Lee, W. H., C. L. Staples, and J. C. Olson, Jr. 1975. *Staphylococcus aureus* growth and toxin survival in macaroni dough and the persistance of enterotoxin in dried foods. J. Food Sci. 40:119-120.
39. Minor, T. E., and E. H. Marth. 1970. Growth of *Staphylococcus aureus* in acidified pasteurized milk. J. Milk Food Technol. 33:516-520.
40. Minor, T. E., and E. H. Marth. 1972. Fate of *Staphylococcus aureus* in cultured buttermilk, sour cream, and yogurt during storage. J. Milk Food Technol. 35:302-306.
41. Minor, T. E., and E. H. Marth. 1971. *Staphylococcus aureus* and staphylococcal food intoxication. A review. I. The staphylococci: Characteristics, isolation, and behavior in artificial media. J. Milk Food Technol. 34:557-564.
42. Minor, T. E., and E. H. Marth. 1972. *Staphylococcus aureus* and staphylococcal food intoxication. A review. II. Enterotoxins and epidemiology. J. Milk Food Technol. 35:21-29.
43. Minor, T. E., and E. H. Marth. 1972. *Staphylococcus aureus* and staphylococcal food intoxication. A review. III. Staphylococci in dairy foods. J. Milk Food Technol. 35:77-82.
44. Minor, T. E., and E. H. Marth. 1972. *Staphylococcus aureus* and staphylococcal food intoxication. A review. IV. Staphylococci in meat, bakery products, and other foods. J. Milk Food Technol. 35:228-241.
45. Minor, T. E., and E. H. Marth. 1976. Staphylococci and their significance in foods. Elsevier Scientific Publishing Company, Amsterdam.
46. Mulhron, F. J. 1975. Nitrates, nitrites, and salt. Notice of proposed rulemaking. Fed. Reg. 49(218):52614-52616.
47. Palumbo, S. A., and J. L. Smith. 1982. Repair of heat-injured *Staphylococcus aureus* 196E on food substrates and additives and at different temperatures. J. Food Protect. 45:458-461.
48. Palumbo, S. A., J. L. Smith, and J. S. Kissinger. 1977. Destruction of *Staphylococcus aureus* during frankfurter processing. Appl. Environ. Microbiol. 34:740-744.
49. Peterson, A. C., J. J. Black, and M. F. Gunderson. 1962. Staphylococci in competition. I. Growth of naturally occurring mixed populations in precooked frozen foods during defrost. Appl. Microbiol. 10:16-22.
50 Pierson, M. D., L. A. Smoot, and N. J. Stern. 1979. Effect of potassium sorbate on growth of *Staphylococcus aureus* in bacon. J. Food Protect. 42:302-304.
51. Raccach, M. 1981. Control of *Staphylococcus aureus* in dry sausage by a newly developed meat starter culture and phenolic-type antioxidants. J. Food Protect. 44:665-669.
52. Reiser, R., D. Conaway, and M. S. Bergdoll. 1974. Detection of staphylococcal enterotoxin in foods. Appl. Microbiol. 27:83-85.
53. Riemann, H., W. H. Lee, and C. Genigeorgis. 1972. Control of *Clostridium botulinum* and *Staphylococcus aureus* in semi-preserved meat products. J. Milk Food Technol. 35:514-523.
54. Scheusner, D. L., and L. G. Harmon. 1973. Growth and enterotoxin production by various strains of *Staphylococcus aureus* in selected foods. J. Food Sci. 38:474-476.
55. Scott, W. J. 1957. Water relations of food spoilage microorganisms. Adv. Food Res. 7:84-127.
56. Smith, J. L., R. C. Benedict, and S. A. Palumbo. 1982. Acid injury in *Staphylococcus aureus* and its prevention by sugars and polyols. Dev. Ind. Microbiol. 23:587-

57. Smith, J. L., R. C. Benedict, and S. A. Palumbo. 1982. Protection against heat injury in *Staphylococcus aureus* by solutes. J. Food Protect. 45:54-58.
58. Smith, J. L., R. C. Benedict, and S. A. Palumbo. 1983. Relationship of water activity to prevention of heat injury in *Staphylococcus aureus*. Lebensm.-wiss. Technol. Accepted Jan. 83.
59. Smith, J. L., R. L. Buchanan, and S. A. Palumbo. 1983. Effect of food environment on staphylococcal enterotoxin synthesis: a review. J. Food Protect. Accepted Dec. 82.
60. Smith, J. L., and S. A. Palumbo. 1978. Injury to *Staphylococcus aureus* during sausage fermentation. Appl. Environ. Microbiol. 36:857-860.
61. Smith, J. L., and S. A. Palumbo. 1980. Inhibition of aerobic and anaerobic growth of *Staphylococcus aureus* in a model sausage system. J. Food Safety 2:221-233.
62. Smith, J. L., and S. A. Palumbo. 1982. Microbial injury reviewed for the sanitarian. Dairy Food Sanit. 2:57-63.
63. Smittle, R. B. 1977. Microbiology of mayonnaise and salad dressings: a review. J. Food Protect. 40:415-422.
64. Stiles, M. E. 1963. The heat inactivation and injury of *Staphylococcus aureus*. Ph. D. thesis, Dept. of Food Technol., Univ. of Illinois, Urbana.
65. Stiles, M. E., and L. D. Witter. 1965. Thermal inactivation, heat injury, and recovery of *Staphylococcus aureus*. J. Dairy Sci. 48:677-681.
66. Surkiewicz, B. F., R. W. Johnston, and J. W. Carosella. 1976. Bacteriological survey of frankfurters produced at establishments under Federal inspection. J. Milk Food Technol. 39:7-9.
67. Tatani, S. R. 1973. Influence of food environments on growth of *Staphylococcus aureus* and production of various enterotoxins. J. Milk Food Technol. 36:559-563.
68. Tatani, S. R. 1976. Thermal stability of enterotoxins in food. J. Milk Food Technol. 39:432-438.
69. Tatani, S. R., R. Y. Lee, W. A. McCall, and W. M. Hill. 1976. Growth of *Staphylococcus aureus* and production of enterotoxins in pepperoni. J. Food Sci., 41:223-225.
70. Thatcher, F. S., J. Robinson, and I. Erdman. 1962. The 'vacuum-packed' method of packaging foods in relation to the production of botulinum and staphylococcal toxins. J. Appl. Bacteriol. 25:120-124.
71. Troller, J. A. 1973. The water relations of food-borne bacterial pathogens. A review. J. Milk Food Technol. 36:276-288.
72. Troller, J. A., and J. V. Stinson. 1975. Influence of water activity on growth and enterotoxin formation by *Staphylococcus aureus* in food. J. Food Sci. 40:802-804.
73. Walker, G. C., and L. G. Harmon. 1966. Thermal resistance of *Staphylococcus aureus* in milk, whey, and phosphate buffer. Appl. Microbiol. 14:584-590.
74. Woodburn, M., T. N. Morita, and S. Z. Venn. 1973. Production of staphyloccal enterotoxins A, B, and C in colloidal dispersions. Appl. Microbiol. 25:825-833.
75. Zehren, V. L., and V. F. Zehren. 1968. Examination of large quantities of cheese for staphylococcal enterotoxin. J. Dairy Sci. 51:635-644.
76. Zehren, V. L., and V. F. Zehren. 1968. Relationship of acid development during cheesemaking to development of staphylococcal enterotoxin A. J. Dairy Sci. 51:645-649.